UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Tuesday, April 08, 2008

MEMORANDUM

Subject: Acute Toxicity Review for EPA Reg. No.: 47371-130/ Formulation HWS-128
DP Barcode: D347812

From: Ian Blackwell, Biologist
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

Through: Karen Hicks, Team Leader
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

To: Velma Noble, PM 31/ Tracy Lantz
Regulatory Management Branch
Antimicrobials Division (7510P)

Applicant: H&S Chemicals Division

FORMULATION FROM LABEL:

| Active Ingredient(s): | % by wt. |
|---|---|
| Didecyl dimethyl ammonium chloride | 5.07 |
| N-Alkyl dimethyl benzyl ammonium chloride | 3.38 |
| Other Ingredient(s): | 91.55 |
| Total: | 100.00 |

1) BACKGROUND: H&S Chemicals (a division of Lonza, Inc.) has submitted a set of five acute toxicity studies to support the precautionary labeling for their product, "Formulation HWS-128". Important matters concerning this submission are:

a) Some of these studies were conducted in 1995 by Product Safety Laboratories. Knowing that these studies were conducted over ten years ago, it is quite possible that these studies have been previously reviewed by the Agency.

b) This submission includes a report titled Acute Toxicity Profile for HWS-128 Use-Solution (MRID Number 472956-01). The background of this report states that the registrant is citing data from other H&S Chemicals products, to amend the label for precautionary statements for the use-dilution of the product. As the registrant wishes to support the use-dilution by citing data from other products, parts of this submission are actually a **Similarity Determination** or **request for bridging** of acute toxicity data. The data citations listed in the report are as follows:

| Study | Cited Product (Registration Number) |
|---|---|
| Acute oral toxicity | 47371-129 |
| Acute dermal toxicity | 47371-129 |
| Acute inhalation toxicity | 47371-131 |
| Primary eye irritation | HWS-128 Use-Solution |
| Primary skin irritation | HWS-128 Use-Solution |
| Dermal sensitization | 47371-129 |

While this report refers to an acute inhalation toxicity study, that report was not actually included in this submission (it was cited). The Chemistry and Toxicology Team (CTT) conducted a search of Agency records for a review of the cited study.

c) According to page 4 of 12 of this report, "HWS-256 is a much more concentrated version of the HWS-128-Use Solution".

d) In addition to some of these studies having been previously reviewed, these studies were reviewed by the Product Science Branch (PSB)/Antimicrobials Division (AD) contractor, Computer Sciences Corporation (CSC).

e) The registrant submitted new (previously unsubmitted) acute toxicity studies to support the primary eye, and primary skin irritation study requirements. They were conducted using the actual use-dilution of the registration product.

f) The dermal sensitization study was conducted using Reg. No. 47371-129. Although Page 4 of MRID Number 472956-01 states: "Citing new study conducted with HWS-256, Reg. No. 47371-129 ...", the cover page lists this study as having been completed in 1995.

2) RECOMMENDATIONS: PSB findings are:

a) The Chemistry and Toxicology Team (CTT) first notes that these studies are submitted and reviewed to support precautionary labeling for the **use-dilution** of Registration Number 47371-130. This precautionary labeling is intended to be displayed on the product label **in addition to** the precautionary labeling of the regular, undiluted version of 47371-130.

b) The Chemistry and Toxicology Team (CTT) allows the registrant to bridge the acute oral toxicity study from 47371-129 to support the acute oral toxicity requirement for 47371-130. This bridging is based upon the following:

i) Registration Number 47371-130 is a dilution (in water) of 47371-129.
ii) The acute toxicity study conducted in on 47371-129 is assigned toxicity category IV. (CTT does not bridge down from toxicity categories I or II.)

c) The Chemistry and Toxicology Team (CTT) bridges the acute dermal toxicity study from Registration Number 47371-129 to support the acute dermal toxicity requirement for 47371-130. Like the bridging of the acute oral toxicity study, this citation and categorization of data is based upon the following:

i) Registration Number 47371-130 is a dilution (in water) of 47371-129.
ii) The acute toxicity study conducted on 47371-129 is assigned toxicity category III.

d) CTT cites a 1/9/1991 Technical Support Section Toxicology Review from the Disinfectants Branch as the review of the acute inhalation toxicity study. That review assigns this (acute inhalation toxicity) study toxicity category III.

e) The primary eye irritation and skin irritation studies are acceptable.

f) CTT was not able to locate the original review of the dermal sensitization study; so, the CSC review of the dermal sensitization study is cited.

The acute toxicity profile for Registration Number 47371-130 is currently:

| Study | MRID Number | Toxicity Category | Status |
|---|---|---|---|
| Acute Oral Toxicity | 472956-02 | IV | Bridged |
| Acute Dermal Toxicity | 472956-03 | III | Bridged |
| Acute Inhalation Toxicity | 414227-01 | III | Cited |
| Primary Eye Irritation | 472956-04 | III | Acceptable |
| Primary Skin Irritation | 472956-05 | IV | Acceptable |
| Dermal Sensitization | 472956-06 | Nonsensitizer | Acceptable/ Cited |

3) LABELING:

a) The Chemistry and Toxicology Team (CTT) notes that the precautionary labeling prescribed in this review is for the **use-dilution** of the product only. That is, this labeling applies to the product once it is diluted in accordance with the product label. It is expected that the product as marketed will contain the original, more stringent, precautionary labeling.

b) The Signal Word (of the diluted product) is Caution. CTT does not know if the PM Team would require a Signal Word for the use-dilution; but, is providing one in just in case.

c) The Precautionary Statements are:

"Harmful if inhaled or absorbed through skin. Causes moderate eye irritation. Avoid contact with skin, eyes or clothing. Avoid breathing vapor or spray mist. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using restroom. Remove and wash contaminated clothing before reuse.

d) The First Aid statements should state:

If in Eyes:

- Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye.
- Call a Poison Control Center or doctor for treatment advice.

If on Skin or Clothing:

- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes.
- Call a Poison Control Center or doctor for treatment advice.

If Inhaled:

- Move person to fresh air.
- If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible.
- Call a Poison Control Center or doctor for treatment advice.

**DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)**

**Product Manager**: 31
**MRID No.**: 472956-02

**Reviewer**: CSC
**Study Completion Date**: November 8, 1995
**Project ID.**: 3655

**Testing Laboratory**: Product Safety Laboratories, East Brunswick, NJ
**Author**: Gary Wnorowski, B.A.

**Quality Assurance (40 CFR §160.12)**: A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that the study meets the requirements of 40 CFR Part 160 with the following exception: "The stability, characterization, identity and verification of the test substance concentration as received and tested are the responsibility of the study sponsor."

**Test Material**: Formulation HWS-256, Lot #: CM7-77 R4-3B / Clear colorless liquid

**Dosage**: 1,000 mg/kg, 1,750 mg/kg, 2,500 mg/kg, and 5,000 mg/kg (administered as received)

**Species**: 40 Rats; Sprague-Dawley derived, albino
**Sex**: 20 Males and 20 Females
**Age**: Young adult [Specific age was not provided]
**Weight**: Males: 184-241 grams; Females: 195-214 grams; at initiation
**Source**: Hilltop Lab Animals, Scottdale, PA
**Housing**: Temperature Range: 68-72°F (i.e., 20-22°C)
Relative Humidity: [Information not provided]
Photoperiod: 12-hour light/dark cycle
**Acclimation**: 10, 11, 15 and 17 days

**Conclusion**:

1. **Acute Oral $LD_{50}$ (mg/kg)**:
   Male and Female Rats: 1,450 mg/kg 95% Confidence Interval: 1,164 to 1,807 mg/kg

   Male Rats: 1,700 mg/kg 95% Confidence Interval: 1,468 to 1,968 mg/kg

   Female Rats: 1,300 mg/kg 95% Confidence Interval: 944 to 1,789 mg/kg

   2. **Toxicity Category**: III **Classification**: ___

**Procedure (Deviations from 870.1100)**:

- No procedure deviations were reported.
- The guidelines state that the most sensitive sex (usually females) should be used. The laboratory tested 5 female and 5 male rats for each dosage level.
- The guidelines specify that females used in the study should be nulliparous and non-pregnant. The laboratory did not specify whether females were nulliparous and non-pregnant.

- The guidelines state that each animal should be between 8 and 12 weeks old. The laboratory specified only that young adult animals were used.
- The guidelines state that the relative humidity of the experimental animal room should be at least 30% and preferably not exceed 70%. The laboratory did not describe the humidity conditions of the experimental animal room.
- The guidelines state that animals should be observed individually at least once during the first 30 minutes after dosing. The laboratory observed animals at least once daily for 14 days.
- Individual body weights of test animals were recorded; however, changes in body weights were not reported.
- The guidelines state that the rationale for initial dose selection, dose progression factor, and follow-up dose levels should be provided. The laboratory did not provide this information.
- The study was conducted using the product, Formulation HWS-256, which contains 15.64% quat. The product, Formulation HWS-128, is the product for which registration is sought and contains 8.45% quat. The study assigned MRID 472956-01 notes that the tested product is a more concentrated version than the product for which registration is sought.

**Results:**

**Reported Mortality**

| 1Dose Level (mg/kg) | Number Dead / Number Tested | | |
|---|---|---|---|
| | Males | Females | Total |
| 1,000 | 0 / 5 | 1 / 5 | 1 / 10 |
| 1,750 | 3 / 5 | 4 / 5 | 7 / 10 |
| 2,500 | 5 / 5 | 5 / 5 | 10 / 10 |
| 5,000 | 5 / 5 | 5 / 5 | 10 / 10 |

**Observations:**

1,000 mg/kg Dose Level (10 animals):

One female died within twenty-two hours of test substance administration. Prior to death, this animal exhibited a hunched posture. Most surviving animals also exhibited a hunched posture as well as an irregular respiration, diarrhea, and/or ano-genital staining. All affected survivors recovered from the above conditions by Day 4 and gained bodyweight over the 14-day observation period.

1,750 mg/kg Dose Level (10 animals):

Three males and four females died within five days of test substance administration. Toxic signs prior to death included nasal discharge, hunched posture, hypoactivity, piloerection, irregular respiration, ano-genital staining, and diarrhea. The surviving animals exhibited similar clinical signs. One surviving male also appeared pale in color between Days 6 and 8. All survivors recovered from the above conditions by Day 9 and gained bodyweight over the 14-day observation period.

2,500 mg/kg Dose Level (10 animals):

All animals died within two days of test substance administration. Toxic signs prior to death included hunched posture, hypoactivity, piloerection, ano-genital staining, and diarrhea.

5,000 mg/kg Dose Level (10 animals):
All animals died within 22 hours of test substance administration. Toxic signs prior to death included hunched posture, hypoactivity, piloerection, irregular respiration, ano-genital staining, diarrhea, and prostration.

**Gross Necropsy Findings:**
1,000 mg/kg Dose Level (10 animals):
Gross necropsy of the decedent revealed discoloration of the lungs and intestines and fluid-filled distention of the stomach. Gross necropsy findings at terminal sacrifice of most survivors were generally unremarkable. In one surviving female, a solid white mass was present on the median and posterior lobes of the lung. Red lung discoloration consistent with euthanasia by $CO_2$ inhalation was noted in all other survivors.

1,750 mg/kg Dose Level (10 animals):
Gross necropsy of the decedents revealed discoloration of the lungs, liver, and gastro-intestinal tract. Gross necropsy findings at terminal sacrifice of the survivors were generally unremarkable. Apart from red lung discoloration consistent with euthanasia by $CO_2$ inhalation, all other tissues and organs appeared normal.

2,500 mg/kg Dose Level (10 animals):
Gross necropsy of the decedents revealed discoloration of the lungs, liver, and gastro-intestinal tract and/or rigor mortis.

5,000 mg/kg Dose Level (10 animals):
Gross necropsy of the decedents revealed discoloration of the lungs, liver, and gastro-intestinal tract and fluid-filled distention of the stomach.

**Statistical Analysis:**
The $LD_{50}$ was calculated by the Litchfield-Wilcoxon Method of Probit Analysis; Litchfield, J.T., F.W. Wilcoxon. J. Pharmacology and Experimental Therapeutics 96:99-115(1949).

**DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)**

**Product Manager**: 31
**MRID No.**: 472956-03

**Reviewer**: CSC
**Study Completion Date**: November 7, 1995
**Project ID.**: 3658

**Testing Laboratory**: Product Safety Laboratories, East Brunswick, NJ
**Author**: Gary Wnorowski, B.A.

**Quality Assurance (40 CFR §160.12)**: A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that the study meets the requirements of 40 CFR Part 160 with the following exception: "The stability, characterization, identity and verification of the test substance concentration as received and tested are the responsibility of the study sponsor."

**Test Material**: Formulation HWS-256, Lot #: CM7-77 R4-3B / Clear colorless liquid

**Dosage**: 2,000 mg/kg (applied as received)

**Species**: 10 Rats; Sprague-Dawley derived, albino
**Sex**: 5 Males and 5 Females
**Age**: Young adult [Specific age was not provided]
**Weight**: Males: 206-231 grams; Females: 208-222 grams; at initiation
**Source**: Hilltop Lab Animals, Scottdale, PA
**Housing**: Temperature: 67-74°F (i.e., 19-23°C)
Humidity: [Information not provided]
Photoperiod: 12-hour light/dark cycle
**Acclimation**: 9 days

**Summary**:

1. **$LD_{50}$ (mg/kg)**: Male and Female Rats: >2,000 mg/kg

2. **The estimated $LD_{50}$ is** greater than 2,000 mg/kg in male and female rats.

3. **Toxicity Category**: III **Classification**: ___

**Procedure (Deviations from 870.1200)**:

- No procedure deviations were reported.
- The guidelines state that, if rats are chosen as the test animal, each animal should be between 8 and 12 weeks old. The laboratory specified only that young adult animals were used.
- The guidelines state that, after completion of the study in one sex, at least one group of five animals of the other sex is dosed to establish that animals of this sex are not markedly more sensitive to the test substance. The laboratory appears to have treated both the male and female groups simultaneously.

- The guidelines specify that females used in the study should be nulliparous and non-pregnant. The laboratory did not specify whether females used in the test were nulliparous and non-pregnant.
- The guidelines state that the relative humidity of the experimental animal room should be 30-70%. The laboratory did not describe the humidity conditions of the experimental animal room.
- Individual body weights of test animals were recorded; however, changes in body weights were not reported.
- The study was conducted using the product, Formulation HWS-256, which contains 15.64% quat. The product, Formulation HWS-128, is the product for which registration is sought and contains 8.45% quat. The study assigned MRID 472956-01 notes that the tested product is a more concentrated version than the product for which registration is sought.

**Results:**

**Reported Mortality**

| Dose Level (mg/kg) | Number Dead / Number Tested | | |
|---|---|---|---|
| | Males | Females | Total |
| 2,000 | 0 / 5 | 0 / 5 | 0 / 10 |

**Observations:**
All animals survived and gained weight. Following test substance application, all rats exhibited clinical signs including nasal and ocular discharge, irregular respiration, hunched posture, hypoactivity, and/or ventral straining. Severe dermal irritation including blanching, hyperkeratosis, and eschar was also present at the dose site of all animals. Apart from the dermal irritation in all animals and ocular irritation noted in one male from Days 8 through 14, all rats recovered from the above clinical signs by Day 5 and appeared active and healthy for the remainder of the 14-day observation period.

**Gross Necropsy Findings:**
Gross necropsy findings at terminal sacrifice were generally unremarkable. Apart from red lung discoloration consistent with euthanasia via $CO_2$ inhalation, all tissues and organs appeared normal.

**DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (OPPTS 870.2400)**

**Product Manager:** 31
**MRID No.:** 472956-04

**Reviewer:** CSC and Ian Blackwell
**Study Completion Date:** November 7, 1995
**Project ID.:** 3665

**Testing Laboratory:** Product Safety Laboratories, East Brunswick, NJ
**Author:** Gary Wnorowski, B.A.

**Quality Assurance (40 CFR §160.12):** A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that the study meets the requirements of 40 CFR Part 160 with the following exception: "The stability, characterization, identity and verification of the test substance concentration as received and tested are the responsibility of the study sponsor."

**Test Material:** HWS Ready-to-Use Lot #: CM7-78 R4-4A / Clear colorless liquid
**Dosage:** 0.1 mL (instilled as received)

**Species:** 6 Rabbits; New Zealand, albino
**Sex:** 4 Males and 2 Females
**Age:** Adult [Specific age was not provided]
**Weight:** [Information not provided (and not required)]
**Source:** Davidson's Mill Farm, South Brunswick, NJ
**Housing:** Temperature: 67-72°F (i.e., 19-22°C)
Humidity: [Information not provided]
Photoperiod: 12-hour light/dark cycle
**Acclimation:** 5 days

**Summary:**

1. **Toxicity Category:** III (minimally irritating to the eye)

2. **Classification:** Acceptable

**Procedure (Deviations from 870.2400):**

- No procedure deviations were reported.
- The guidelines state that the age of the test animals should be reported. The laboratory specified only that adult animals were used.
- The guidelines state that the humidity of the caging conditions should be reported. The laboratory did not describe the humidity of the caging conditions.
- The study was conducted using the product, HWS Ready-to-Use, which contains 0.0630% quat. The product, Formulation HWS-128, is the product for which registration is sought and contains 8.45% quat (when undiluted). A use solution of Formulation HWS-128 (when prepared according to label directions) contains 0.066% quat.

**Results:**
All animals appeared active and healthy. Apart from the eye irritation noted below, there were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

No corneal opacity or iritis was noted during the study. One hour after test substance instillation, conjunctivitis was noted in all treated eyes. The incidence and severity of irritation decreased with time. All animals were free from ocular irritation by 72 hours.

The 24-hour Maximum Mean Total Score of HWS Ready-to-Use was 3.3. The Maximum Mean Total Score of HWS Ready-to-Use was 6.7.

**Incidence of Irritation**

| Time Post Instillation | No. of Animals Testing "Positive" / No. of Animals | | | Severity – Mean Score |
|---|---|---|---|---|
| | Corneal Opacity | Iritis | Conjunctivitis | |
| 1 hour | 0 / 6 | 0 / 6 | 6 / 6 | 6.7 |
| 24 hours | 0 / 6 | 0 / 6 | 5 / 6 | 3.3 |
| 48 hours | 0 / 6 | 0 / 6 | 3 / 6 | 1.3 |
| 72 hours | 0 / 6 | 0 / 6 | 0 / 6 | 0 |

**Individual Scores for Ocular Irritation**

| Observations | Rabbit No. 7664 | | | | Rabbit No. 7665 | | | | Rabbit No. 7666 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hours After Treatment | | | | | | | | | | | |
| | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 |
| I. Corneal Opacity | 0 | 0[1] | 0 | 0 | 0 | 0[1] | 0 | 0 | 0 | 0[1] | 0 | 0 |
| II. Iris | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| III. Conjunctivae | | | | | | | | | | | | |
| A. Hyperemia | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 2 | 1 | 1 | 0 |
| B. Chemosis | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| C. Discharge | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |

[1]2% fluorescein sodium used to verify the absence of corneal opacity.

**Individual Scores for Ocular Irritation**

| Observations | Rabbit No. 7667 | | | | Rabbit No. 7668 | | | | Rabbit No. 7669 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hours After Treatment | | | | | | | | | | | |
| | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 | 1 | 24 | 48 | 72 |
| I. Corneal Opacity | 0 | 0[1] | 0 | 0 | 0 | 0[1] | 0 | 0 | 0 | 0[1] | 0 | 0 |
| II. Iris | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| III. Conjunctivae | | | | | | | | | | | | |
| A. Hyperemia | 2 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 1 | 1 | 0 |
| B. Chemosis | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| C. Discharge | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |

[1]2% fluorescein sodium used to verify the absence of corneal opacity.

**DATA REVIEW FOR PRIMARY DERMAL IRRITATION TESTING (OPPTS 870.2500)**

**Product Manager:** 31
**MRID No.:** 472956-05

**Reviewer:** CSC and Ian Blackwell
**Study Completion Date:** November 7, 1995
**Project ID.:** 3666

**Testing Laboratory:** Product Safety Laboratories, East Brunswick, NJ
**Author:** Gary Wnorowski, B.A.

**Quality Assurance (40 CFR §160.12):** A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that the study meets the requirements of 40 CFR Part 160 with the following exception: "The stability, characterization, identity and verification of the test substance concentration as received and tested are the responsibility of the study sponsor."

**Test Material:** HWS Ready-to-Use
Lot #: CM7-78 R4-4A / Clear colorless liquid

**Dosage:** 0.5 mL (applied as received)

**Species:** 6 Rabbits; New Zealand, albino
**Sex:** 3 Males and 3 Females
**Age:** Adult [Specific age was not provided]
**Weight:** [Information not provided (and not required)]
**Source:** Davidson's Mill Farm, South Brunswick, NJ
**Housing:** Temperature: 66-73°F (i.e., 19-23°C)
Humidity: [Information not provided]
Photoperiod: 12-hour light/dark cycle
**Acclimation:** 13 days

**Summary:**

1. **Toxicity Category:** IV (slightly irritating to skin)

2. **Classification:** Acceptable

**Procedure (Deviations from 870.2500):**

- No procedure deviations were reported.
- The guidelines state that the age of the test animals should be reported. The laboratory specified only that adult animals were used.
- The guidelines state that the humidity of the caging conditions should be reported. The laboratory did not describe the humidity of the caging conditions.
- The study was conducted using the product, HWS Ready-to-Use, which contains 0.0630% quat. The product, Formulation HWS-128, is the product for which registration is sought and contains 8.45% quat (when undiluted). A use solution of Formulation HWS-128 (when prepared according to label directions) contains 0.066% quat.

**Results:**

All animals appeared active and healthy. Apart from the dermal irritation noted below, there were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior.

One hour after patch removal, very slight erythema was noted at 4 treated sites, and one animal exhibited very slight edema. Although at 24 hours, irritation persisted at only one site, the incidence of irritation increased at 48 and 72 hours with a very slight to well defined erythema evident at three sites. The incidence and severity of irritation decreased thereafter. Although desquamation was present at 4 of 6 treated sites between Days 7 and 10, all animals were free from erythema and edema by Day 10.

The Primary Dermal Irritation Index for HWS Ready-to-Use was calculated to be 0.7. [Only scores for observations made during 1-72 hours were used in this calculation.]

**Incidence of Irritation**

| Time After Patch Removal | No. of Animals Testing "Positive" / No. of Animals Tested | | Severity of Irritation–Mean Score |
|---|---|---|---|
| | Erythema | Edema | |
| 1 hour | 4 / 6 | 1 / 6 | 0.9 |
| 24 hours | 1 / 6 | 0 / 6 | 0.2 |
| 48 hours | 3 / 6 | 1 / 6 | 0.9 |
| 72 hours | 3 / 6 | 1 / 6 | 0.9 |
| Day 7 | 2 / 6 | 1 / 6 | 0.5 |
| Day 10 | 0 / 6 | 0 / 6 | 0.0 |

**Individual Dermal Irritation Scores**

| Animal No. | Sex | Erythema / Edema | | | | | |
|---|---|---|---|---|---|---|---|
| | | Time After Patch Removal | | | | | |
| | | 1 hour | 24 hours | 48 hours | 72 hours | Day 7 | Day 10 |
| 7548 | M | 1 / 1 | 1 / 0 | 2 / 1 | 2 / 1 | 1 / 1[1] | 0 / 0[1] |
| 7549 | F | 1 / 0 | 0 / 0 | 1 / 0 | 1 / 0 | 1 / 0[1] | 0 / 0[1] |
| 7550 | M | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 |
| 7551 | F | 1 / 0 | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 |
| 7552 | M | 0 / 0 | 0 / 0 | 1 / 0 | 1 / 0 | 0 / 0[1] | 0 / 0[1] |
| 7553 | F | 1 / 0 | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0[1] | 0 / 0[1] |
| **Total** | | 4 / 1 | 1 / 0 | 4 / 1 | 4 / 1 | 2 / 1 | 0 / 0 |
| **Mean** | | 0.7 / 0.2 | 0.2 / 0.0 | 0.7 / 0.2 | 0.7 / 0.2 | 0.3 / 0.2 | 0.0 / 0.0 |

[1]Desquamation present at dose site.

**Summary of Primary Dermal Irritation (PDI) Scores[1]**

| | Time After Patch Removal | | | | | |
|---|---|---|---|---|---|---|
| | 1 hour | 24 hrs | 48 hrs | 72 hrs | Day 7 | Day 10 |
| **Erythema** | 0.7 | 0.2 | 0.7 | 0.7 | 0.3 | 0.0 |
| **Edema** | 0.2 | 0.0 | 0.2 | 0.2 | 0.2 | 0.0 |
| **TOTAL (PDI)[2]** | 0.9 | 0.2 | 0.9 | 0.9 | 0.5 | 0.0 |

[1]Average values for six rabbits.

[2]PDI = Average Erythema + Average Edema

**DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)**
(BUEHLER METHOD)

**Product Manager**: 31
**MRID No.**: 472956-06

**Reviewer**: CSC and Ian Blackwell
**Study Completion Date**: November 8, 1995
**Project ID.**: 3659

**Testing Laboratory**: Product Safety Laboratories, East Brunswick, NJ
**Author**: Gary Wnorowski, B.A.

**Quality Assurance (40 CFR §160.12)**: A Quality Assurance statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that the study meets the requirements of 40 CFR Part 160 with the following exceptions: "1. The stability, characterization, identity and verification of the test substance concentration as received and tested are the responsibility of the study sponsor. 2. The stability, uniformity of mixture and verification of concentration of DNCB in its carriers were not determined."

**Test Material**: Formulation HWS-256, Lot #: CM7-77 R4-3B / Clear colorless liquid

**Positive Control Material**: 1-Chloro-2,4-Dinotrobenzene (DNCB)
(Historical data – performed concurrently with test)

| | | |
|---|---|---|
| **Species**: | 35 Guinea pigs; Hartley, albino | |
| **Sex**: | Range-Finding: | 10 Animals [sex not specified] |
| | Test Group: | 10 Males |
| | Naïve Control Group: | 5 Males |
| | Naïve Control Group Rechallenge: | 5 Males |
| | Positive Control Group: | 10 Males |
| | Positive Naïve Control Group: | 5 Males |
| **Age**: | Young adult [Specific age was not provided] | |
| **Weight**: | 303-428 grams at initiation | |
| **Source**: | Davidson's Mill Farm, South Brunswick, NJ | |
| **Housing**: | Temperature: 66-72°F (i.e., 19-22°C) | |
| | Humidity: [Information not provided] | |
| | Photoperiod: 12-hour light/dark cycle | |
| **Acclimation**: | 6 days | |

**Method**: Buehler Method

**Summary**:

1. **Based on these findings and on the evaluation system used, Formulation HWS-256 is not considered to be a contact sensitizer.**

2. **Classification**: Acceptable

**Procedure (Deviations from 870.2600):**

- No procedure deviations were reported.
- The guidelines state that the relative humidity of the experimental animal room should be 30-70%. The laboratory did not describe the humidity conditions of the experimental animal room.
- The guidelines recommend using a minimum of 20 animals in the treatment group and at least 10 animals as controls. The laboratory used 10 animals in the treatment group and 5 animals as controls.
- The laboratory only graded erythema, and not edema, although the guidelines require that as a minimum, the erythema and edema must be graded.
- The guidelines state that the age of the test animals should be reported. The laboratory specified only that young adult animals were used.
- The study was conducted using the product, Formulation HWS-256, which contains 15.64% quat. The product, Formulation HWS-128, is the product for which registration is sought and contains 8.45% quat. The study assigned MRID 472956-01 notes that the tested product is a more concentrated version than the product for which registration is sought.

**Procedure:**

Preliminary Irritation Testing: On April 19, 25, May 19 and 24, 1995, a group of animals was used to determine the highest non-irritating concentration (HNIC) of the test substance prior to the [induction and] challenge dose. The fur was removed by clipping the dorsal area and flanks of each guinea pig. This area was divided into four test sites (two sites on each side of the midline) on each animal. The test substance was used as received or diluted with distilled water to yield concentrations of 100%, 75%, 50%, 25%, 10%, 5%, 3.5%, 2%, and 1% w/w. Each concentration was applied to a test site using an occlusive 25 mm Hilltop Chamber. The sites were wrapped with non-allergenic Durapore adhesive tape. After 6 hours of exposure, the chambers were removed. Twenty-four hours after application, each site was evaluated for local reactions (erythema) according to a scoring system provided in the laboratory report.

From these results, the HNIC (the highest concentration that produced responses in 4 guinea pigs no more severe than two scores of 0.5 and two scores of zero) was established and used for challenge. Based on these findings, the HNIC selected for the challenge phase was a 3.5% w/w solution in distilled water.

Preparation and Selection of Animals: On the day before initiation, the fur of a group of animals was removed by clipping the dorsal area and flanks. After clipping and just prior to initiation, the animals were weighed and the skin was checked for any abnormalities. Only healthy animals without pre-existing skin irritation were selected for test. Animals were re-clipped prior to each dose.

Induction Phase: Once each week for three weeks, 0.4 mL of a 5% w/w solution of the test substance in distilled water was applied to the left side of each test animal using an occlusive 25 mm Hilltop Chamber. The chambers were secured in place and wrapped with non-allergenic Durapore adhesive tape to avoid dislocation of the chambers and to prevent evaporation. After the 6-hour exposure period, the chambers were removed and the test sites were gently wiped with water and a clean towel to remove any residual substance. Twenty-four and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system.

Challenge Phase: Fifteen days after the last induction, a challenge dose was applied to a naïve site on the right side of each animal, using the procedures described above. The HNIC of the test substance (3.5% w/w solution in distilled water) was used for the challenge phase.

These sites were evaluated for a sensitization response (erythema) at 24 and 48 hours after the challenge application according to the scoring system. In addition to the test animals, 10 guinea pigs from the same shipment were maintained under identical environmental conditions and were treated with the HNIC of the test substance at challenge only. These animals constituted the "naïve control" group.

Rechallenge Phase: Due to the presence of faint erythema at three test sites 48 hours after the challenge dose, a rechallenge was conducted six days after primary challenge. An additional group of 5 animals was placed on test to serve as a naïve control group for rechallenge. Four tenths of a milliliter of the test substance at its HNIC (3.5% w/w solution in distilled water) was applied to a naïve site on the right side of each test and naïve control guinea pig, using the procedure above. The sites were evaluated for a sensitization response (erythema) at 24 and 48 hours after the challenge application according to the scoring system.

Historical Positive Control: The procedures used in this study were validated using 1-Chloro-2,4-Dinitrobenzene (DNCB) as a positive control substance. This test was conducted with Hartley strain albino guinea pigs following induction and challenge procedures similar to those described above. The selected induction dose of the historic positive control was 0.4 mL of 0.08% DNCB in 80% aqueous ethanol. The selected HNIC of DNCB was a 0.04% w/w solution in acetone.

**Results:**

Induction Phase:

*Test Animals (5% w/w solution of test substance in distilled water)*: Very faint to severe erythema (0.5-3) was noted at all test sites during the induction phase. Overall, the incidence and severity of irritation increased with each successive application. Eschar was also evident at two sites at 48 hours after the second dose. In several instances, a decrease in irritation was noted after the dose sites were relocated to an adjacent area for the third induction.

*Positive Control Animals (0.08% DNCB in 80% aqueous ethanol)*: Very faint to moderate erythema (0.5-2) was noted at all positive control sites during the induction phase. Severe erythema was evident at one site 48 hours after the third induction. Overall, the incidence and severity of irritation increased with each successive application.

Challenge Phase:

*Test Animals (3.5% w/w solution of test substance in distilled water)*: Very faint erythema (0.5) was noted at six of ten sites 24 hours after challenge dose. At 48 hours, faint erythema (1) was evident at three sites and 5 animals exhibited very faint erythema (0.5).

*Naïve Control Animals (3.5% w/w solution of test substance in distilled water)*: Very faint erythema (0.5) was noted at four naïve control sites 24 hours after the challenge dose and at all five sites at 48 hours.

*Positive Control Animals (0.04% DNCB in acetone)*: Three of ten positive control animals exhibited signs of a sensitization response (faint to moderate erythema [1-2]) 24 hours after challenge. At 48 hours, similar indications persisted at these sites and severe erythema (3) was evident in one additional animal. Eschar or edema was also noted at two sites following the challenge dose.

*Positive Naïve Control Animals (0.04% DNCB in acetone)*: Very faint erythema (0.5) was noted for two positive naïve control sites 24 hours after challenge. Irritation cleared from both affected sites by 48 hours.

Rechallenge Phase:

*Test Animals (3.5% w/w solution of test substance in distilled water)*: Very faint erythema (0.5) was noted at two sites 24 and 48 hours after rechallenge.

*Naïve Control Animals (3.5% w/w solution of test substance in distilled water)*: Very faint erythema (0.5) was noted at four naïve control sites 24 hours after rechallenge. Irritation persisted in three affected sites through 48 hours.

**Sensitization Response Indices (Erythema)**

| | Incidence of Positive Response[1] | | Severity[2] | |
|---|---|---|---|---|
| | 24 hours | 48 hours | 24 hours | 48 hours |
| **Test Animals – Challenge** | 0 / 10 | 3 / 10 | 0.30 | 0.55 |
| **Naïve Control Animals – Challenge** | 0 / 5 | 0 / 5 | 0.40 | 0.50 |
| **Test Animals – Rechallenge** | 0 / 10 | 0 / 10 | 0.10 | 0.10 |
| **Naïve Control Animals – ReChallenge** | 0 / 5 | 0 / 5 | 0.40 | 0.30 |

[1]Animals with scores greater than 0.5.
[2]Sum of the erythema scores divided by the number of animals evaluated.

**Test Animal Group Skin Reaction Scores**

| | Induction | | | | | | Challenge | | Rechallenge | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment Phase** | 1 | | 2 | | 3 | | | | | |
| **Concentration** | 5% | | 5% | | 5% | | 3.5% | | 3.5% | |
| **Hours[1]** | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 |
| **Animal No. / Sex** | | | | | | | | | | |
| **Test Group** | | | | | | | | | | |
| **2058 / M** | 0.5 | 0 | 2 | 2 | 2 | 3 | 0.5 | 0.5 | 0.5 | 0.5 |
| **2059 / M** | 1 | 0.5 | 1 | 1 | 2 | 3 | 0 | 0 | 0 | 0 |
| **2060 / M** | 1 | 1 | 0.5 | 0.5 | 3 | 3 | 0 | 1 | 0 | 0 |
| **2061 / M** | 1 | 1 | 2 | 3[2] | 2 | 2 | 0.5 | 0.5 | 0.5 | 0.5 |
| **2062 / M** | 0.5 | 0.5 | 2 | 3[2] | 1 | 1 | 0.5 | 0.5 | 0 | 0 |
| **2063 / M** | 1 | 0.5 | 1 | 3 | 1 | 1 | 0.5 | 0.5 | 0 | 0 |
| **2064 / M** | 1 | 0 | 1 | 3 | 1 | 1 | 0.5 | 1 | 0 | 0 |
| **2065 / M** | 0 | 0 | 1 | 3 | 1 | 2 | 0.5 | 1 | 0 | 0 |
| **2066 / M** | 0.5 | 0 | 0.5 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| **2067 / M** | 1 | 0 | 1 | 3 | 2 | 1 | 0 | 0.5 | 0 | 0 |
| **Naïve Control Group** | | | | | | | | | | |
| **2068 / M** | -- | -- | -- | -- | -- | -- | 0 | 0.5 | 0.5 | 0.5 |
| **2069 / M** | -- | -- | -- | -- | -- | -- | 0.5 | 0.5 | 0.5 | 0.5 |
| **2070 / M** | -- | -- | -- | -- | -- | -- | 0.5 | 0.5 | 0 | 0 |
| **2071 / M** | -- | -- | -- | -- | -- | -- | 0.5 | 0.5 | 0.5 | 0 |
| **2072 / M** | -- | -- | -- | -- | -- | -- | 0.5 | 0.5 | 0.5 | 0.5 |

[1]Hours after induction dose.
[2]Eschar present at dose site.